MEMBRANE POTENTIAL AND RESISTANCE OF THE STARFISH EGG BEFORE AND AFTER FERTILIZATION [1]

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Unequal distribution of ions between the interior and exterior is characteristic of living cells. In many thus far studied the concentration of potassium is much higher in the interior while the concentrations of sodium and chloride are lower. This ionic asymmetry is associated with a potential difference across the plasma membrane which is approximately related to the relative concentration of potassium (Höber, 1945; Hodgkin, 1951) according to the Nernst equation:

$$E = \frac{RT}{nF} \ln \frac{(K^+)i}{(K^+)_0} \qquad \text{or at } 20^\circ \text{ C.,} \qquad E \text{ (in mv.)} = -58 \log \frac{(K^+)i}{(K^+)_0}.$$

Membrane potentials have been recorded from many cells (Hodgkin, 1951; Grundfest, 1955), including some whose internal potassium concentration is known, by means of a fine, saline-filled, microcapillary (Gelfan, 1927, 1931; Ling and Gerard, 1949) inserted through the cell surface. The magnitude of this potential in different cells ranges from 50 to 100 mv., inside negative. This indicates an internal excess of potassium approximately 9 to 50 times the external concentration, and is in approximate accord with the observed values in specific cases in which potassium concentration has been determined.

Several investigators (Gelfan, 1931; Rothschild, 1938; Kamada and Kinosita, 1940) had, many years ago, reported that they could find no potential difference across the membrane of echinoderm eggs.[6] Interest in this problem has sharpened recently because of two new factors. In the first place a number of workers (Scheer,

[1] This work was reported at the General Scientific Session of the Marine Biological Laboratory in 1955 (Grundfest, Kao, Monroy and Tyler, 1955; and Tyler, Monroy, Kao and Grundfest, 1955). We wish to thank the Director and Staff of the MBL for the facilities placed at our disposal.

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[6] In a brief report Taylor and Whitaker (1926) mention experiments on eggs of the sea urchin *Clypeaster rosaceus* showing a potential difference, inside negative, of about 1 mv., which would be very low in comparison with other kinds of cells that have been investigated.

Monroy, Santangelo and Riccobono, 1954; Furshpan, 1955; Kao, 1955) have independently made similar observations in several varieties of marine eggs, using modern recording equipment and stable KCl-filled (Kamada and Kinosita, 1940; Nastuk and Hodgkin, 1950) microcapillary electrodes. In the second place, convincing data have become available (Rothschild and Barnes, 1953) showing that at least for the eggs of the sea urchin *Paracentrotus lividus*, the potassium content in the aqueous phase of the egg is 21 times higher than in sea water, while the internal sodium and chloride concentrations are, respectively, about $\frac{1}{9}$ and $\frac{1}{7}$ of those in sea water. It would therefore seem likely that in the sea urchin egg a membrane potential of about 80 mv., inside negative, should be observed.

The persistent failure to find a membrane potential prompted a re-examination of this problem with certain technical refinements which provide definitive verification of the entry of a microelectrode into the cell, as well as measurements of the resistance and capacity of the membrane. Parallel experiments with microinjection (Tyler and Monroy, 1955) helped to elucidate and overcome difficulties encountered in attempts to pierce the cell membrane of echinoderm eggs. A potential difference was thereupon found to exist across the membrane of *Asterias* eggs. Its magnitude was found to be somewhat lower than would be expected on the basis of the high internal K^+, which was also determined in these experiments. As in other kinds of cells that have been investigated, the membrane potential difference changes reversibly on changing the external K^+ concentration.

Although, as will be shown below, it is unlikely that penetration by the microelectrode had been attained in earlier work, several observers (Rothschild, 1938; Scheer *et al.*, 1954; Furshpan, 1955) have reported that eggs could be fertilized while apparently impaled. Fertilization was also successful in the present experiments with the electrode truly inside the egg. The effects of fertilization on the potential and on the electrical constants of the membrane were therefore also studied.

Methods

Tyler and Monroy (1955) carried out experiments attempting microinjection of fluids into eggs of *Arbacia, Echinarachnius* and *Asterias*. A smaller number of experiments were performed in the present series in an effort to penetrate eggs of *Arbacia punctulata* with microelectrodes. Confirming the experience of Chambers, (Pandit and Chambers, 1932, and personal communication) in both cases it was found that piercing the surface is difficult. Microelectrodes or micropipettes which appear to have penetrated, in actuality only carry the membrane before them even to the extent of creating a tunnel (as Dan, 1943, has also observed) as the microcapillary travels through the diameter of the egg. This was clearly revealed in the microinjection experiments of Tyler and Monroy (1955), in which it was observed that the membrane could form a tight sleeve around the inserted pipette, the latter then appearing to be within the cytoplasm of the egg. However, injected fluid (KCl-NaCl solutions) would simply expand this sleeve and flow out into the surrounding medium rather than into the egg. Eggs of *Asterias forbesii* behaved similarly, as illustrated in Figure 1, but in view of their larger size (average diameter, 146 μ) these were chosen in preference to eggs of *Arbacia* for further investigation. Penetration of these eggs was accomplished by the technique of jarring the preparation by a light tap on the table. This sudden vibration was especially ef-

fective after the indented plasma membrane had formed a tight sleeve around the electrode (as in Figure 1e) and was allowed to remain in this condition for a short while. Figure 2 illustrates eggs with one or two electrodes that have penetrated into the cytoplasm.

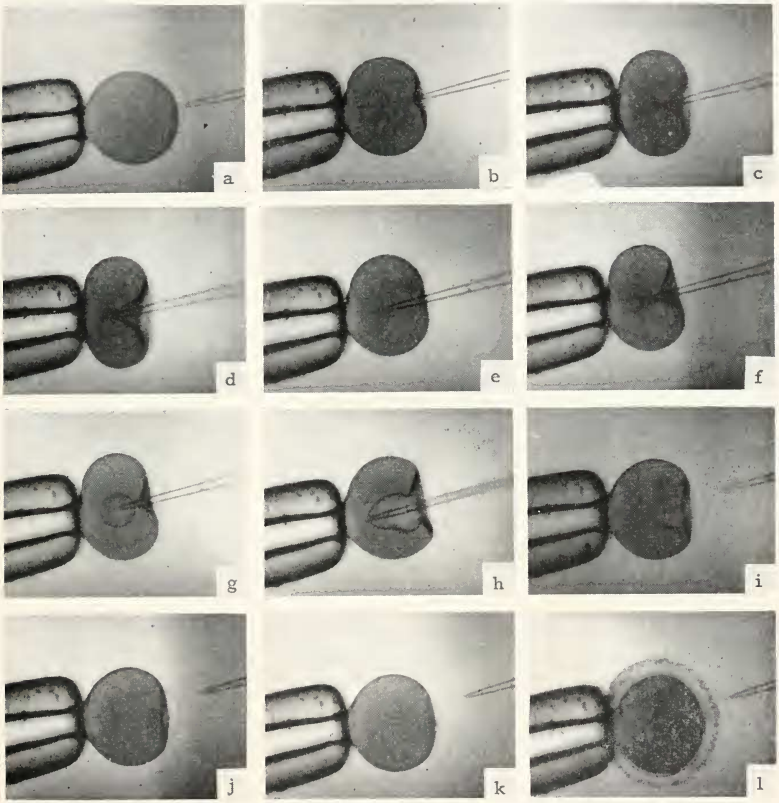

FIGURE 1. Photomicrographs showing apparent entry of a micropipette into an egg of *Asterias forbesii* held by a "sucking" pipette. Magnification, 104 ×. The micropipette, filled with isotonic NaCl–KCl solution containing chlorphenol red, is pushed through the vitelline membrane and indents the underlying surface (plasma membrane) forming a large conical depression (*d*). After about two minutes the depression closes over the pipette (*e*) and the latter appears to be within the cytoplasm. However, injection of fluid (*f* to *h*) shows that the walls of the depression had formed a tight sleeve around the shaft of the pipette. The fluid expands this sleeve and stretches the vitelline membrane, flowing out through the latter. Upon removal of the pipette (*i* to *k*) the remaining fluid is expelled as the egg rounds-up within some two minutes. The same egg with a fertilization membrane elevated at two minutes after fertilization is shown in *l*.

The experiments were carried out primarily on unfertilized eggs. A few measurements were made on eggs with germinal vesicles or fertilized prior to impalement by microelectrodes. The eggs were obtained from spontaneously shedding animals. They were kept at temperatures of 18 to 20° C. until used, one to five hours after shedding. The experiments were carried out mostly at about 25° C. and all the eggs used appeared to be normal. All those so tested, as well as parallel samples, were fertilizable. Some batches of eggs were stored at about 10° C. but these underwent spontaneous activation on transfer to the room temperature and were, therefore, not used in the experiments.

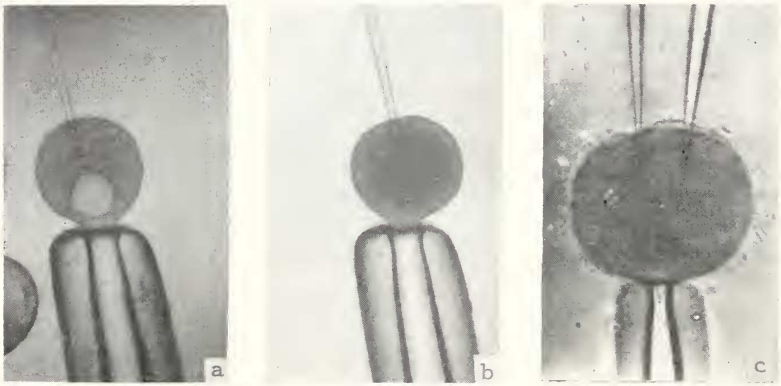

FIGURE 2. Photomicrographs of eggs of *Asterias forbesii* held on "sucker" and impaled on microelectrodes. Magnification: *a* and *b*, 116×; *c*, 200×. *a*, an egg with intact germinal vesicle; *b*, another after dissolution of the germinal vesicle; *c*, an egg fertilized after insertion of two microelectrodes. The tips of the microelectrodes are not visible.

All the impaled eggs, in the experiments involving fertilization, had undergone dissolution of the germinal vesicle, and were in various stages of the maturation divisions. Sperm were diluted in a solution of 10^{-3} molar Versene [7] in sea water, since the latter improves the fertilizing power of dilute sperm suspensions (Tyler, 1953). The sperm were introduced by means of a capillary pipette at a distance several millimeters from the egg. The time at which sperm were seen to approach the egg, as well as the time of formation of the fertilization membrane were noted for correlation with the measurements of the membrane potential.

Experimental arrangement

Sea water containing eggs was placed (Fig. 3A) on a transilluminated lucite plate mounted on a mechanical stage under a binocular microscope. The sea water was in continuity with one end of a sea water-filled tunnel, into the other end of which was inserted an Ag-AgCl reference electrode. One or two microelectrodes (tip diameters less than 0.5 μ), each individually carried in a micromanipulator, ap-

[7] Versene is the trade name (Bersworth Chemical Co.) of ethylene diamine tetraacetic acid.

proached diagonally from above at a small angle. Opposite was another manipulator which held a glass suction pipette. This device (Tyler, 1955b), modified from the elastimeter of Mitchison and Swann (1954), was very useful for holding the egg fixed gently but firmly at one pole while the microelectrodes were pressed against the other (Figs. 1 and 2). The polished tip of the "sucker," somewhat smaller than the diameter of the egg, dipped into the sea water containing the eggs. The other end was flexibly coupled to a vertical glass tube which could be raised or lowered by a rack and pinion movement. The system was filled with sea water. By maneu-

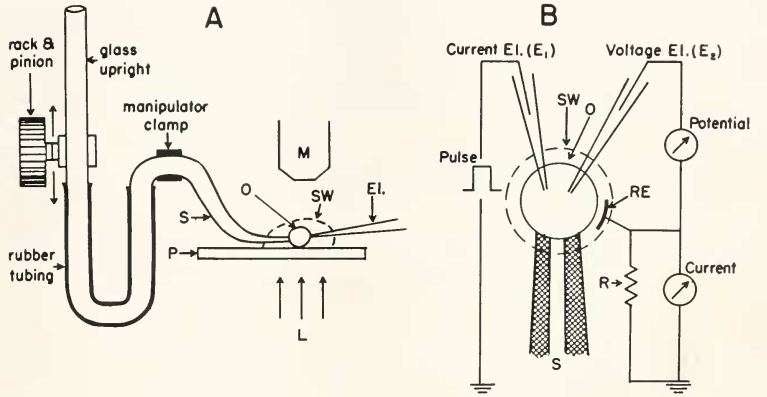

FIGURE 3. Diagrammatic illustration of the experimental arrangement. *A:* The mechanical and optical set-up. The egg (O) is shown (enlarged) lying in a drop of sea water (SW) on a lucite plate (P) which is illuminated from below (L) and observed through a microscope (M). The sucker (S) has a rack and pinion for raising or lowering the vertical tube. The electrodes (E) are inserted into the egg at the pole opposite that held in the sucker. *B:* The electrical arrangement. The two microelectrodes ($El._1$ and $El._2$) are shown in the egg (O). $El._1$ is the current electrode fed through a pulse generator. $El._2$ is connected to one grid of the potential recording amplifier. The other grid connects with the fluid (SW) as well as with a resistor (R) through the reference electrode (RE). Across R is the amplifier measuring the current in the pulse. When a single microelectrode ($El._2$) was used, RE was grounded.

vering the mechanical stage, any desired egg in the drop could be brought to the vicinity of the tip of the sucker. Lowering the upright created sufficient negative pressure to take up and hold the egg firmly against the tip. Manipulation was carried out under 80 or 160 × magnification.

Electrical measurements

Determination of the membrane potential and the resistance and capacity of the membrane constituted the electrical measurements. For the former a single microelectrode, drawn prefilled with 3 *M* KCl (Kao, 1954), was sufficient. This was connected to a high impedance negative capacity input amplifier [8] and a cathode ray

[8] Designed by Mr. E. Amatniek, electronic engineer at the Dept. of Neurology, Columbia University.

oscillograph. The external medium was grounded through the Ag-AgCl reference electrode, or in some cases the "sucker" was itself made the reference system. The standard sensitivity employed was 20 mv./cm. deflection on the face of the oscillograph tube so that changes in potential as low as two to three mv. could be detected. Visual observation was supplemented by photography of the trace.

Determination of the electrical constants of the membrane required passage of a square pulse current through the membrane and the measurement of the potential difference created by this current across the resistance and capacity of the membrane (Fig. 3B). For this purpose two microelectrodes were inserted into the eggs (Fig. 2c). One of these was connected to a pulse generator delivering 12 or

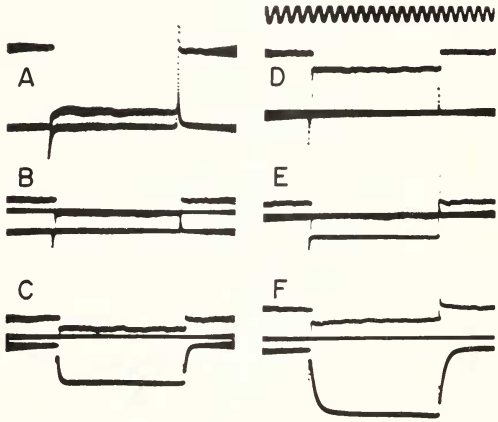

FIGURE 4. Current and voltage pulses as recorded before and after entry of two microelectrodes into the egg. *Left:* A large current pulse (*A*, upper trace) caused only transient capacitative artifacts in the voltage trace (below) as long as the electrodes were outside the egg. *B, C:* The current pulse was reduced. *B*, before and *C*, after penetration of the electrodes. A third trace, which represents the zero level at the time of entry, is seen at its correct position in *C*. The membrane potential, about −20 mv., has brought the voltage trace down. The square pulse of current is reflected in a membrane *IR* drop with retarded onset and decay. *Right:* Another egg. *D:* The current pulse caused only the capacitative artifact on the voltage trace. *E:* When the two electrodes were pressed against the egg, the voltage trace also recorded a deflection with rapid onset and decay. However, the steady potential was zero. *F:* A few seconds later, the electrodes had penetrated the egg, causing the characteristically slowed onset and decay of the voltage trace. A membrane potential of about −30 mv. is also seen. Time scale in msec., upper right.

30 msec. pulses of controllable amplitude, synchronized in rate and time with the sweep of the oscillograph. The external reference electrode was connected to a resistance, the other end of which was grounded, as was the return of the stimulator. An amplifier across the resistor recorded the *IR* drop in the latter and the current, *I*, through the membrane was calculated from this measurement. The sensitivity of the current trace on the oscillograph was 1 mv./cm. and with a 1 megohm resistor for the *IR* drop this amplitude of deflection corresponded to 0.001 μA.

The second microelectrode led, as before, to another amplifier, but in this case recording the membrane potential differentially, the second grid being connected to the indifferent electrode and the high end of the resistor. The potential change of this amplifier during the square pulse thus represented the IR drop across the membrane in series with the resistance of the fluid. Since the resistance of the latter was small compared with that of the membrane, it was neglected. From the knowledge of I obtained in the current record, R of the membrane could be computed. This was transformed to the specific membrane resistance R_M (ohm-cm.^{2}) by multiplying by the surface area of the egg (average diameter = 146 μ; surface = 6.7 $\times$

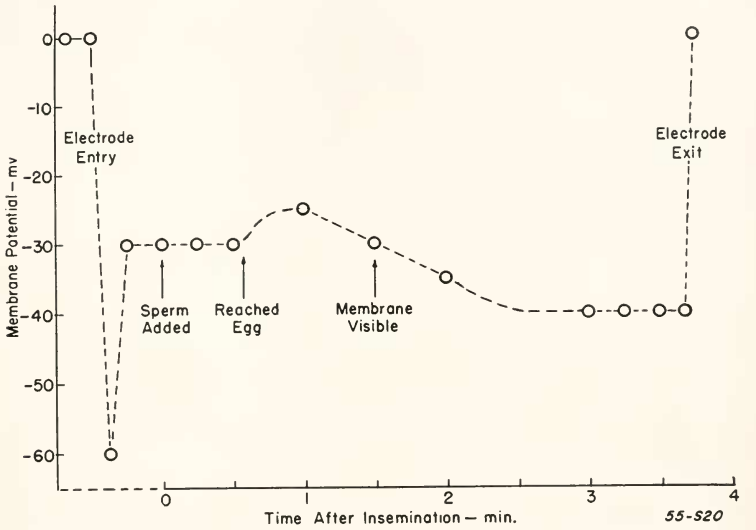

FIGURE 5. Membrane potential of Asterias egg and its changes on fertilization. Entry of the electrode into the egg caused sudden appearance of -60 mv. membrane potential, which decreased to -30 mv. rapidly. The time scale has its origin at the time sperm were added to the sea water. Within 30″ sperm were seen to have contacted the egg and at this time the previously steady potential decreased by 5 mv. Subsequently the potential again increased and remained steady at -40 mv. until the electrode was removed. Absence of drift in the system is indicated by the return of the voltage trace to the initial value.

10^{-4} cm.^{2}). The time constant (τ) when the rise and fall of the voltage trace (Fig. 4C, F) had reached 67% of the final value was measured from the records. The membrane capacity (C_M) was determined in μF/cm.^{2} from the relation $\tau = R_M C_M$.

Certain precautions have to be taken in experiments of this type. The current electrode must be non-polarizable in the range of currents used for the measurements. This was checked at the start and end of each experiment. Secondly, applied current must be rather low. With a microelectrode tip of 0.5 μ diameter, 1 μA flow represents a current density at the tip of about 500 A/cm.^{2} which might lead to

heating and perhaps breaking of the electrode. Furthermore, a flow of 1 μA through the whole surface area of the egg membrane is equivalent to a current of 1.5 mA/cm.^{2} which is a high density, at least for the membranes of excitable tissues.

The use of the two microelectrodes and a current pulse served the additional important purpose of providing unequivocal evidence of the penetration of both electrodes into the egg (Fig. 4). When these electrodes were in the fluid the record of the voltage trace differed radically from the trace of the current pulse (Fig. 4A, B, D). The former showed only rapid short-lived deflections of opposite sign at the beginning and end of the applied pulse. These are attributable to capacitative

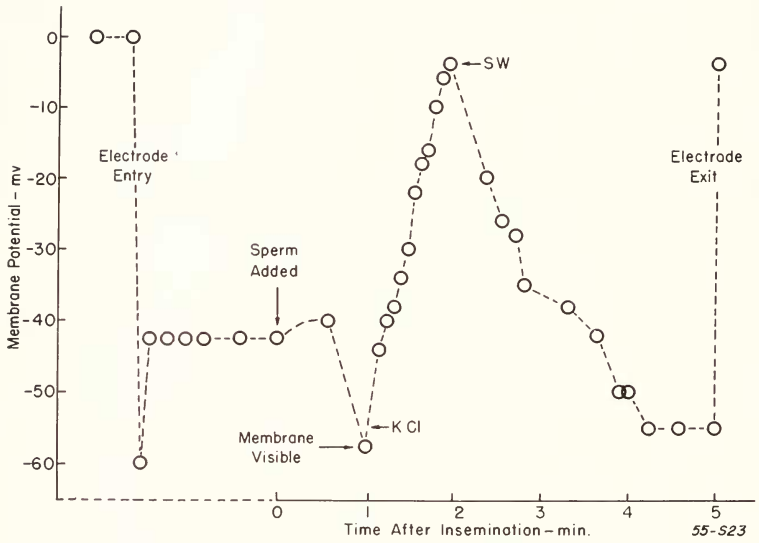

FIGURE 6. Depolarizing effect of externally applied KCl and its reversibility. The sequence up to one minute on the time scale is similar to that of Figure 5. When the membrane potential of the fertilized egg had reached its maximum value, the sea water was largely replaced with isotonic KCl. This caused rapid and almost complete depolarization, which was reversed on washing out the KCl with sea water. A drift in the amplifier of 4 mv. negative had changed the base line slightly.

coupling between the electrodes. During the major portion of the applied pulse, the voltage trace remained essentially at zero potential, reflecting the low resistance of the sea water and the consequently low IR drop across it. On occasion, when both electrodes were simultaneously pressed firmly against the egg, the voltage trace showed a large deflection which would be expected if the current path now included a high resistance formed by the surface of the egg membrane. However, this voltage record was characterized (Fig. 4E) by its faithful reproduction of the form of the current pulse, indicating that the recording electrode was not across

the capacity of the membrane. When penetration occurred the voltage pulse changed in form characteristically, rising and falling more slowly than the current trace (Fig. 4C, F). This behavior, in view of our original uncertainty as to the existence of a potential difference across the egg membrane, proved valuable initially in definitely establishing the entry of the microelectrodes into the cytoplasm.

Ionic content of Asterias eggs

Measurements of the internal ionic milieu of *Asterias* eggs were not found in the literature. A sample of over two million eggs was therefore subjected to analysis by flame photometry. The procedure is detailed below in conjunction with the data obtained.

Results

Membrane potential

In nearly all the eggs studied no potential accompanied apparent penetration by the electrode, but one or more taps on the table always caused the sudden appearance of a potential difference with the internal electrode negative, except in a number of eggs which cytolyzed as the electrode appeared to enter. The failure to obtain the

Table I

Steady potential difference observed across the membrane of unfertilized eggs

P.D. in mv., inside negative	10	15	20	25	30	40	45	50
Number of eggs (Total 24)	3	2	5	1	8	3	1	1

In one additional egg, fertilized prior to penetration of the electrode, the membrane p.d. was initially −40 mv., and increased to −48 mv.

In one other egg only a p.d. of +10 mv. was obtained.

potential initially was probably due to extensibility of the egg membrane. Jarring probably caused penetration of the membrane during the resultant vibration. The potential, upon penetration, reached values up to 60 mv., apparently instantaneously, then rapidly declined to a lower steady value (Figs. 5, 6). The steady value of the membrane potential ranged in different experiments from a low of 10 mv. to a high of 50 mv. (Table I), with the majority of eggs showing potentials of 20 to 30 mv.

The larger initial value probably reflects more nearly the true potential difference momentarily disclosed as the fine tip of the microelectrode broke through the egg membrane. The subsequent release of tension of the latter, as it rounds up and moves farther onto the electrode, could create an imperfect seal of the membrane around the shaftlet and lead to partial short circuiting of the full potential. In most experiments the potential remained steady at the lower value as long as the electrode was left in the egg. Withdrawal was accompanied by an abrupt return of the oscillograph trace to the zero potential level. There seemed to be no consistent difference in the value of the steady potential if two electrodes were inserted simultaneously or sequentially. The number of experiments of this type was too

small and the scatter of potentials too great to employ this method for calculating the possible magnitudes of leaks around the electrode.

Two eggs included in Table I, through which relatively high currents (0.5 to 1.0 μA) were later passed, cytolyzed in the course of the experiments. Five cytolyzed spontaneously, and two that had an intact germinal vesicle cytolyzed on subsequent penetration of this structure. In all cases cytolysis resulted in disappearance of the membrane potential. In three cases the potential disappeared with no observable cytolytic effects. Movement of the tip then again disclosed the membrane potential. These can be interpreted as eggs in which the cytoplasm had become sealed off from the electrode (Chambers, 1922) by a precipitation membrane (Heilbrunn, 1927, 1952; *cf.* Costello, 1932). In a few other experiments, all done with the same microelectrode, penetration was indicated at first by a small positive potential (about 10 mv.) which in all but one case reversed to negativity. The positive potential may have been due to increase of the electrode resistance by plugging of the tip as this pressed into the egg membrane. With the grid current of the amplifier about 10^{-11}A and positive, a shift of + 10 mv. would be caused by insertion of a resistance of 10^9 ohms. Another possibility is that the electrode had penetrated the egg, but had been sealed off from the cytoplasm while some leakage remained around the shaft. The internal negativity would then register as positivity on pickup by the external electrode.

Membrane potential upon fertilization

Eight eggs were fertilized while impaled. Insemination was done usually at least 5 minutes after impalement in order to ascertain that the membrane potential was steady and that the egg was not undergoing cytolytic changes. When spermatozoa were seen to have reached the impaled egg (about 15 to 30 seconds after insemination) the membrane potential suddenly decreased from its previously steady value. This change amounted to 5 to 10 mv., and was temporary (Figs. 5, 6). The membrane potential then began to increase gradually, eventually attaining a magnitude greater than the former steady value, and in some cases as large as that momentarily seen during entry of the electrode. This increased internal negativity persisted during the subsequent period of observation which, for the present series of experiments, was not longer than 5 minutes. The new steady value of potential, 5 to 20 mv. higher than before fertilization, was attained in 1 to 2.5 minutes, which was also the time at which the fertilization membrane had become distinctly elevated.

Ionic content of Asterias eggs

Estimate of the magnitude of the membrane potential to be expected requires knowledge of the ionic concentration in the egg. Determinations were therefore made of the K and Na content of *Asterias* eggs. The procedure was as follows:

A 100-ml. suspension of unfertilized eggs was prepared in sea water. From this, a one-ml. sample was removed by means of a wide-mouth (2.5 mm.) pipette. It was diluted 20-fold and a one-ml. portion used for counting the number of eggs. During these procedures precautions were taken to keep the suspension of eggs distributed as uniformly as possible. The final one-ml. diluted sample contained

1096 eggs. Therefore the 99 ml. of the original suspension contained 2.17×10^6 eggs. The eggs of the latter suspension were allowed to settle, and most of the fluid was drawn off. The remainder was then centrifuged for 15 minutes at $1500 \times g$, in graduated centrifuge tubes. The packed eggs measured 6.1 ml., and above them was an additional gelatinous, opalescent layer of 2.1 ml. representing the material of the gelatinous coat of the egg. Supernatant fluid was withdrawn to leave a total volume of 10 ml. of packed eggs, gelatinous layer and sea water. The eggs lost during the procedure were determined from counts of aliquots of the

TABLE II

Measurements of diameters of eggs of Asterias forbesii

Number of eggs	1	3	1	1	2	2	1	3	Total: 14
Average of the two diameters (μ)	140	142.5	143.8	145	146.3	147.5	148.8	150	Average: 145.9

supernatants. Their number was 2128 or less than 0.1% of the total in the packed eggs. To the 10 ml. volume of packed eggs and supernatant, and separately to an equal volume of supernatant fluid, were added 10 ml. of sulphuric acid. The two preparations were allowed to stand overnight, transferred with distilled water washings to digestion flasks and boiled for about 4 hours, one ml. of 30% H_2O_2 being added to help clarify the material. Both preparations were then transferred to 100-ml. volumetric flasks and made up to that volume in distilled water. The original samples had thereby been diluted 10-fold. These were analyzed for K^+ and Na^+ by flame photometry.[9]

TABLE III

Determinations of K and Na content of eggs of Asterias forbesii

	Potassium	Sodium
(1) mM in 10 ml. of suspension containing 2.168×10^7 eggs	0.656	2.40 to 2.61
(2) mM in 10 ml. of supernatant	0.205	4.00 to 4.32
(3) mM in 6.47 ml. of supernatant	0.133	2.59 to 2.79
(4) mM in 3.53 ml. of eggs [(1)–(3)]	0.523	−0.19 to −0.18
(5) mM/ml. eggs	0.148	

To calculate the content of these ions in the eggs it was necessary to determine the egg volume, exclusive of interstitial fluid. The diameters of 14 eggs, in which the difference between diameters at right angles was less than 4%, were measured, with the results shown in Table II. The flame photometric determinations and calculated values of K and Na content are given in Table III.

On the basis of an average diameter of 146 μ the volume of each egg is 1.63×10^{-6} cm.^{3} and that of the total number in the suspension is 3.53 ml. The latter value is 58% of the volume (6.1 ml.) of the packed eggs after the low speed centrifugation, and is in reasonable agreement with the value to be expected from the packing of spheres, plus a small allowance for adherent jelly coat. The 10-ml.

[9] We are indebted to Dr. James Green of Rutgers University and to Dr. George Scott of Oberlin College for the analyses.

specimen containing eggs therefore was composed of 35.3% eggs and 64.7% interstitial fluid (including gelatinous coat material). From the measurements of the parallel sample of supernatant fluid the 6.47 ml. of the supernatant in the egg sample contained 0.133 mM K^+. The 3.53 ml. of eggs therefore contained 0.523 mM or 0.148 mM/ml. of eggs. This figure is about 15 times the K^+ concentration of sea water (0.01 mM/ml.). On the basis that the eggs contain approximately 75% water by weight and 80% by volume, the K^+ concentration becomes 0.185 mM/ml.

TABLE IV

Electrical constants of Asterias eggs

Expt. No.	Conditions	Steady membrane potential (mv.)	Maximum applied current (μA)		r_m (ohms)	R_M (ohm-cm.^{2})	τ (msec.)	C_M (μF/cm.^{2})
			Outward	Inward				
A. Maximum currents not exceeding .01 μA								
25	unfertilized	—*	.006	.006	4.0×10^6	2680	1.28	0.48
	fertilized	—	.006	.006	3.6	2410	1.25	0.52
26	unfertilized	−30	.006	.006	4.97	3330	1.92	0.58
27	unfertilized	−15	.005	.01	3.7	2430	1.04	0.43
28	unfertilized	−15	.005	.005	5.8	3880	2.75	0.71
	fertilized	−50	.005	.005	5.8	3880	1.6	0.41
B. Higher maximum currents								
7	unfertilized	−10	0.10	0.06	5.05×10^5	368		
8a	unfertilized	−10	0.10	—	11.	737		
b	unfertilized	−10	0.15	—	8.6	574		
11	unfertilized	−20	0.25	—	2.6	172		
6	unfertilized	−20	0.40	—	2.0	133		
13	unfertilized	−30	0.4	0.4	1.07	71.6		
1a	unfertilized	−30	1.0	1.0	2.6	174		
b	unfertilized		1.0	1.0	2.9	194		
14	unfertilized	—*	1.5	1.5	2.5×10^4	16.8		

* Potential not measured because of amplifier drift.

of the water in the eggs. This corresponds fairly closely with the value of 0.210 mM/mg. of water found in eggs of the sea urchin *Paracentrotus lividus* (Rothschild and Barnes, 1953).

The supernatant contained about the same amount of sodium as does sea water, but the potassium concentration (0.02 mM/ml.) was twice that of sea water (Table II). For the calculation given above it was assumed that the extra K^+ derived from gelatinous material of the eggs, some of which remained in the supernatant. On the other hand, if this potassium had leaked out of the eggs during preparation for analysis, the initial concentration of the ion in the eggs would have been 0.168 mM/ml. of eggs and 0.210 mM/ml. of the water in the eggs.

Similar calculations for the Na content of the starfish eggs, using either the values of 2.40 and 4.00 mM/10 ml. of egg suspension and supernatant, respectively,

or 2.61 and 4.32 gave a slightly negative value (− 0.2 mM per 3.53 ml. eggs) for this ion. In view of the high content of Na in sea water and therefore in the interstitial fluid of the egg suspension, the value for Na is much more sensitive to errors in determination of egg volume than is that of K. For example, if the actual egg-volume were 13% greater than determined, the calculated Na content would be zero while that of K would be 0.17 mM/ml. of the water in the eggs. We may therefore conclude that the Na concentration in *Asterias* eggs is less than one-twentieth, while the K concentration is between 17 and 21 times, the values found in sea water.

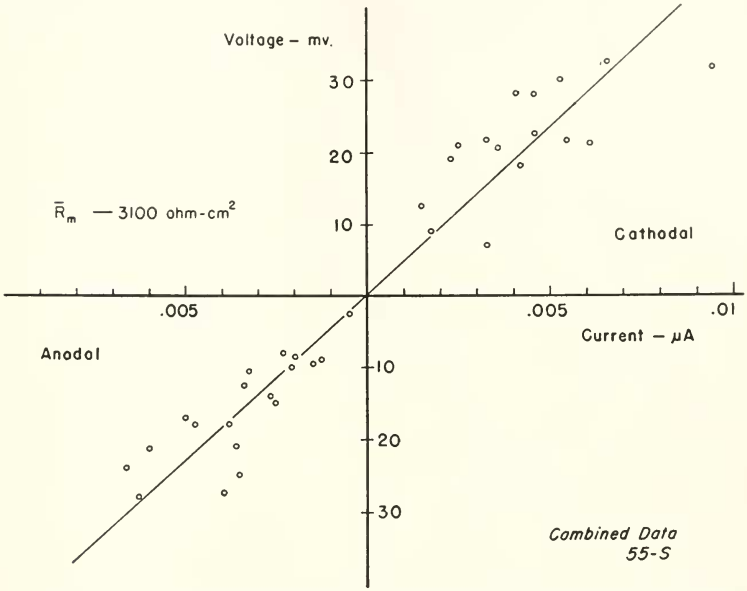

FIGURE 7. Membrane current-membrane voltage relation in four Asterias eggs. Two of the six experiments represent measurements done after fertilization. The slope of the straight line drawn through the combined data is the average resistance (r_m) and yields $R_M = 3100$ ohm-cm². *Anodal* signifies outward current and *cathodal*, inward.

Change in membrane potential on increasing external K^+

Four experiments served to test and demonstrate the sensitivity to K^+ of the potential difference across the egg membrane, but the relation between the external K^+ and the potential was not studied quantitatively. Within a few seconds after isosmotic KCl was added to the sea water surrounding an impaled egg the membrane potential decreased (Fig. 6) and reached almost complete depolarization. The K^+-rich solution was then replaced with sea water and the initial value of the membrane potential was again restored. Fertilized and unfertilized eggs responded

in the same manner. The technical arrangement did not permit rapid washing out of the excess KCl and this probably accounts for the slower return of the initial membrane potential.

Membrane resistance and capacity

These electrical constants of the membrane were determined in 10 eggs with the square pulse technique described earlier. However, in six the maximum current densities were rather high (0.5 to 1.5 μA) and the results were probably affected

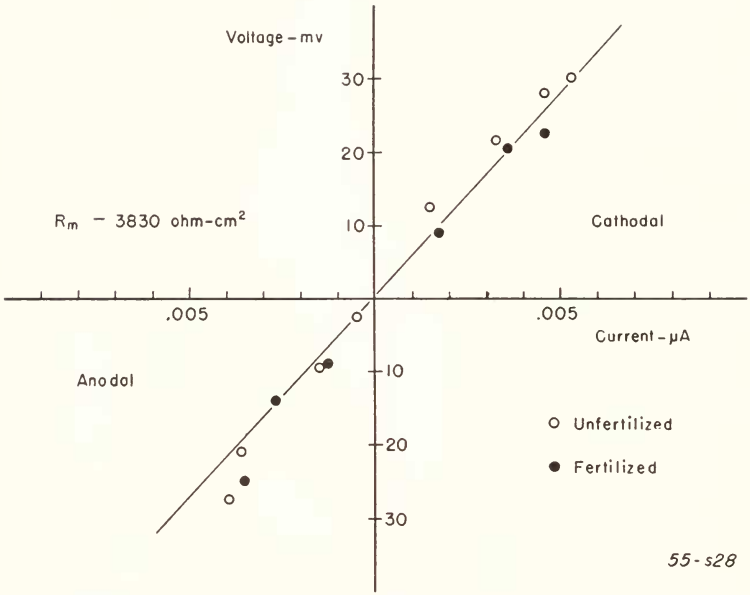

FIGURE 8. Membrane resistance before and after fertilization. The current-voltage relation of the same egg before and after fertilization.

by this condition. In six experiments with four eggs (two eggs were studied after fertilization as well as before) the maximum current of the square pulse was limited to 0.01 μA and these results are shown in Table IVA and Figures 7 and 8. The average membrane resistance was 3100 ohm-cm.[2] In the two eggs measured after the fertilization membrane was elevated there was no significant change in resistance (Fig. 8; Table IVA). In the six experiments employing high current densities (0.5 to 1.5 μA) the values of R_M were markedly smaller (Table IVB). No explanation will be attempted at present for this effect. Over the range of current densities used in all the experiments the relation between membrane voltage and current was linear. Assuming a specific resistance of 100 ohm-cm. (approximately

three times that of sea water; Cole and Cole, 1936a, 1936b) for the cytoplasm, the droplet constituting the interior of the egg would have a resistance of about 8×10^3 ohms, surrounded by a membrane with a resistance several orders higher than this in magnitude.

Determination of the time constant (τ) and of the membrane capacity (C_M) was only approximate, because the oscillographic records of the membrane voltage change were made on too slow a time base. The average capacity (0.52 μF cm.^{2}) is somewhat smaller than, but of the same order of magnitude as, the 1.1 μF cm.^{2} obtained by Cole and Cole (1936a) with eggs of *Asterias forbesii*, and 0.7 to 2.7 μF cm.^{2} listed by Cole and Curtis (1950) for unfertilized eggs of other marine animals.

Discussion

The original object of this investigation was to seek an explanation for the reported absence of a membrane potential in some echinoderm eggs. On the basis of Rothschild and Barnes' (1953) finding that sea urchin eggs contain 21-fold higher concentration of K^+ than does sea water it was to be expected that a membrane potential of about 80 mv. ought to be present. A membrane potential has now been found in *Asterias* eggs. Its magnitude, at least 60 mv. under what we believe to be the optimum condition, is smaller by about 15 to 20 mv. than might be expected on the basis of the values (17 : 1 to 21 : 1) that we obtained for the ratio of K^+ in *Asterias* eggs to that in sea water. A similar discrepancy is usually found in nerve and muscle fibers (Hodgkin, 1951 : Grundfest, 1955).

The reversible depolarization of the egg membrane in response to increasing the external K^+ agrees with the behavior of other cells (Hodgkin, 1951), but the quantitative relation between potential and K^+ concentration was not tested in the present experiments. Another point of similarity relates to the low internal Na^+ concentration. *Asterias* eggs contain too little Na^+ for accurate measurement under the experimental conditions employed. Rothschild and Barnes (1953) found a concentration of 52 mM/kg. water in eggs of *Paracentrotus lividus* as compared with 485 for sea water, and the Na^+ concentration of various excitable cells is also considerably lower than in the fluid surrounding them (Hodgkins, 1951). It is thereby likely that an active transport mechanism exists in echinoderm eggs as it apparently does in other types of cells (*cf.* Brown and Danielli, 1954).

Rothschild (1938), and Kamada and Kinosita (1940) had considered, but rejected, the possibility that failure to obtain a membrane potential might be due to failure of electrode to penetrate the egg. Their decision was based on the apparent entrance of injection fluid into the egg. Furshpan (1955) believed that because in many experiments he had pushed the electrode clear through the egg, it must have been in the cytoplasm at some stage in the process and therefore considered his results to demonstrate absence of a membrane potential. However, as has been noted by Dan (1943) and by Tyler and Monroy (1955), the micropipette can readily tunnel through the egg, without entering the cytoplasm; the distended plasma membrane on one side simply joins that on the other and both are then perforated without injury to the egg which can later close the tunnel. It is pertinent to quote in this connection the remarks of Chambers (1922, p. 189) :

Pushing a pipette, especially a comparatively large one, into an egg cell frequently causes the surface of the cell to become invaginated and thus forms a deep pocket. The tip of the pipette, even if it should finally break through the surface, is apt to become separated from the protoplasm of the interior by the formation of a new surface film continuous with the original surface of the cell.

Chambers' conclusion was confirmed by Tyler and Monroy (1955, and illustrated in Fig. 1) and in the present experiments. It is now also well known, particularly from the work of Heilbrunn (1927, 1952; *cf.* Costello, 1932), that when the cytoplasm of eggs of marine animals, or of other cells, is brought in contact with Ca-containing solution a surface precipitation reaction occurs. The formation of such a precipitation membrane around the tip of the pipette might have been responsible for the lack of a membrane potential in the experiments of Rothschild (1938) in which injection tests indicated penetration. The absence of a potential in the experiments of Gelfan (1931), Kamada and Kinosita (1940) and Scheer *et al.* (1954) can also be attributed to failure of penetration or possible formation of a precipitation membrane.

A small positive potential was observed by Gelfan (1931) when the microelectrode presumably penetrated the germinal vesicle of the *Asterias* eggs. In our experiments, confirming Chambers (1921), puncture of the germinal vesicle invariably led to cytolysis of the egg. The sudden disappearance of the membrane potential when the germinal vesicle was impaled and cytolysis resulted, indeed served as additional verification of penetration in the experiments reported in the present paper. As noted, and discussed earlier, small positive potentials were occasionally observed in these experiments.

Although the absence of a membrane potential in echinoderm eggs reported by earlier observers is explained in the light of the present experiments, there remains the finding (Kao, 1955) that eggs of the killer minnow, *Fundulus*, do not exhibit a membrane potential. The precautions using two microelectrodes and an applied pulse, were also employed in those experiments to ascertain penetration of the egg membrane. However, the ionic composition of *Fundulus* eggs is unknown and the explanation for this different finding must remain in abeyance.

For the most part measurements of the membrane potential have been carried out on cells from tissue aggregates. Some data are, however, available for unicellular organisms. These are of interest not only because they provide a rather closer analog to eggs than do tissue components, but also because they, too, reflect the effects of improvements in technique. Telkes (1931) reported that amoebae have a membrane potential of 10 to 30 mv., inside negative. Buchthal and Péterfi (1937) found only small variable potentials of either sign (up to 3 mv.). Later, however, Wolfson (1943) succeeded in recording a membrane potential of up to 90 mv. in *Chaos chaos*. Dr. S. Crain, of the Department of Neurology, Columbia University (personal communication) has obtained similar values for the membrane potential in *Chaos chaos* and *Paramaecium*. As in eggs, it is difficult to penetrate the cell membrane and Wolfson used the device of applying negative pressure on the electrode, sucking the amoeba onto the shaftlet and eventually rupturing its cell membrane. Tauc (1953) found a membrane potential of 80 to 100 mv. in the plasmodium of a myxomycete. As in the *Asterias* egg, the high initial value decreased subsequent to penetration.

Effects of fertilization on the membrane potential

A second objective of these experiments was to examine whether or not changes in membrane potential accompany the events of fertilization. Péterfi and Rothschild (1935), using two small external electrodes placed on opposite sides of the surface of the frog egg, reported (p. 875) that "there are strong indications that the attachment of the spermatozoon to the egg results in an action potential being propagated over the egg surface, the action potential being characterized by having no recovery phase." Scheer *et al.* (1954), although they could not obtain a steady membrane potential with an electrode apparently inserted into eggs of *Paracentrotus lividus* and *Arbacia lixula,* reported transient potential differences upon fertilization. These changes consisted of a series of rapid pulses of irregular size, ranging from about 2 to 5 mv. They began at the time that the first visible reaction (cortical change) to the sperm was observed, persisted during the period of egg contraction and gradually disappeared. In the case of *Paracentrotus* eggs the pulses were much less frequent (often only one or two) than in *Arbacia* (as many as fifty). Scheer *et al.* (1954) point out that these changes are not strictly comparable to the action potentials of nerve and muscle. Furshpan (1955) saw no potential changes upon fertilization of eggs of the sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus,* but as in the case of the other observers, neither was a steady membrane potential obtained with these eggs. Similarly Kamada and Kinosita (1940), using an "internal" electrode found no change upon fertilization, nor a "resting" potential in eggs of the sea urchin *Strongylocentrotus pulcherrimus.* An attempt to examine possible changes in membrane potential upon fertilization was also made recently by Allen, Lundberg and Runnström (1955) with external electrodes in contact through sea water in agar with the ends of a capillary tube in which a sea urchin egg was elongated and fertilized. They found no shift in potential but concluded that the technique proved inadequate for this problem.

The present series of experiments disclose that in eggs of *Asterias forbesii,* at least, there is a change in the membrane potential beginning with a sudden decrease when spermatozoa are seen to have made contact with the egg. The initial decrease of membrane potential is converted to an increase which reaches its maximum and steady value when the fertilization membrane is raised. These changes in membrane potential appear to develop smoothly without the occurrence of the pulses recorded by Scheer *et al.* (1954) with a capacitatively coupled amplifier.

The time course of the initial decrease in potential was not determined accurately. The optical system did not permit identification of the moment of sperm entry into the egg, and unfortunately photographic recording of the oscillograph traces was not done at sufficiently frequent intervals to define accurately the initial portion of the membrane potential change. However, visual observation at 1 per second sweeps, which was routinely done, indicated that the decrease took less than 10 seconds to reach its maximum value.

More detailed experiments will be required to establish whether or not the observed electrical changes in *Asterias* eggs are associated in time with the optical changes which accompany fertilization. The color change which passes over the surface of the sea urchin egg within a few seconds after the attachment of the sperm (Runnström, 1928) is a propagated response with a total conduction time of about 20 seconds at 18° C. (Rothschild and Swann, 1949). Birefringence of the surface

disappears in about the same time (Monroy and Montalenti, 1947). These events therefore have about the same time course as the decrease and subsequent beginning of the return of the membrane potential to its former steady amplitude.

Studies on the rate at which block to polyspermy is established in sea urchin eggs (Rothschild and Swann, 1950, 1951, 1952; Rothschild, 1953, 1954) have led to the conclusion that there is a fast reaction which passes over the surface of the egg in about two seconds and that reduces the chances of refertilization by a factor of 20. This is followed by a slower change which in about 60 seconds reduces to zero the probability of a successful sperm-egg collision. The wave of explosion of the "Harvey-Moser granules" (Harvey, 1911, p. 523; Moser, 1939) takes about 15 seconds to traverse the surface of an echinoid egg from the point of attachment of the sperm (Endo, 1952). The time course of these events is about the same in the starfish egg judging from the fact that the fertilization membrane becomes visibly elevated at one to two minutes. As noted earlier, the new maximum of membrane potential is attained at about the time that the fertilization membrane is clearly visible.

A distinction must, however, be made in correlating the electrically and optically observed effects. The latter are initiated at the site of entry of the sperm and are slowly propagated from there. The former are recorded with an electrode inside a sphere of small diameter and this condition operates against the likelihood of observing discretely localized membrane potential change. If the electrical changes observed during fertilization have their basis in localized changes of the membrane, these are probably electronically averaged in the actual recording and would not reveal clearly a spreading electrical change which might accompany the propagated evolution of the optically observed phenomena.

The nature of the membrane events which lead to the electrical changes observed after fertilization can only be speculated upon. Potential change in excitable tissues is associated with change in membrane permeability and/or altered ionic flux (Bernstein, 1912; Hodgkin and Katz, 1949; Fatt and Katz, 1951; Hodgkin and Huxley, 1952; Eccles, Fatt and Koketsu, 1954). In the more complex bioelectric generators of frog skin or the gastric mucosa, changes in the transport respectively of Na^+ (Ussing, 1954; Kirschner, 1955) and Cl^- (Hogben, 1955) are involved. At least in the former case, metabolic activity and hormonal factors play an important role. Whether or not the electrical changes which occur upon fertilization are associated with known metabolic changes (*cf.* Brachet, 1947; Runnström, 1949; Tyler, 1955a) is not at present clear. Changes in ionic permeability such as might be indicated by the electrical data probably do occur. However, the time course of the K^+ accumulation is not known. Monroy-Oddo and Esposito (1951) have reported that sea urchin eggs gain K^+ upon fertilization, and such accumulation might account for the late increase in the membrane potential. Malm and Wachmeister (1950), on the other hand, report a slight decrease in potassium content, and a considerable increase in sodium, of sea urchin eggs upon fertilization. They attribute this to the fertilization membrane being permeable to the ions in the surrounding sea water, so that analyses after fertilization would show an increase in ions present in high concentration in sea water and an apparent decrease in ions like potassium previously accumulated by the egg. Shapiro and Davson (1941) had also reported no significant change in potassium content in sea urchin eggs upon fertilization. They noted, too, that both unfertilized and fertilized eggs lost potassium slowly

(1.5 to 8% in two hours) on standing in sea water, and that eggs in K^+-enriched (5 ×) artificial sea water accumulated K^+. In experiments with radioactive cations Brooks (1939) noted an increase in the accumulation of radiosodium upon fertilization in eggs of *Urechis caupo*. E. L. Chambers *et al.* (1948) reported that the rate of exchange of K^{42} increased 7 to 13 times upon fertilization in eggs of *Strongylocentrotus purpuratus* and *Arbacia punctulata*. They considered (see also E. L. Chambers, 1949) that only 20 per cent of the K is readily exchangeable in the unfertilized egg and 85 to 100 per cent in the fertilized egg. The rate of exchange of "freely diffusible" K is therefore concluded to be two to three times more rapid in the fertilized egg. While there are some evident differences in the results of the various experiments cited, the indications are that there are likely to be changes in permeability to certain ions, occurring upon fertilization, that may correlate with the changes in electrical potential.

On the other hand, the electrical changes might rather be due to alteration in the mechanical properties of the egg membrane. The large potential initially seen on penetrating the unfertilized egg was about equal in magnitude to the largest steady potential attained after fertilization. The decline from the initial value has been interpreted as being caused by imperfect sealing of the plasma membrane around the shaftlet of the microelectrode. It is therefore possible that the initial decrease upon fertilization indicates a further loosening of the seal and that the subsequent rise of membrane potential only reflects formation of a better seal. The various physical changes initiated in the fertilization reaction and discussed above might well be implicated in an alteration of the membrane seal around the electrode.

The electrical constants of the egg membrane

The measurements of membrane resistance and capacity were in the present experiments subsidiary to the use of the square pulse technique for ascertaining penetration of the egg surface by the microelectrodes. Therefore the results are chiefly indicative of the orders of magnitude of these values, subject to a more extensive study. However, the measurements can throw some light on the more detailed interpretation of the membrane potential and will be discussed largely in this context.

The unfertilized egg

Most cells thus far adequately studied have membrane resistances in the range of 1000 ohm-cm.^{2} However, the values range from as low as 0.1 ohm-cm.^{2} (rostral membrane of the electoplaque of the eel; Keynes and Martins-Ferreira, 1953) to as high as 20,000 ohm-cm.^{2} (activated *Fundulus* egg; Kao, 1955). The membrane resistance of *Asterias* eggs (average: 3,100 ohm-cm.^{2}) indicates that ionic transport is rather low across this membrane. In a tabulation of the membrane resistance of various cells, Cole and Curtis (1950) give an estimate that the membranes of both sea urchin and starfish eggs have infinite resistance. Earlier, however, Cole and Cole (1936a) pointed out that the method of measurement was not appropriate for determinations of membrane resistance. A 2% change in the estimate of relative cell volumes to that of external fluid would have led to a calculated value of membrane resistance as low as 25 ohm-cm.^{2} Rothschild (1938) stated that while his attempts to measure membrane resistance of *Echinus* eggs were unsatisfactory, the

value was probably no higher and perhaps lower than 10^4 ohm-cm.^{2} Furshpan (1955) estimated that the resistance which the sea urchin egg interposed in the microelectrode circuit of his experiments was about 1 megohm. From the surface area of these eggs (diameter, 75 μ; area 1.8×10^{-4} cm.^{2}) he calculated the membrane resistance as 180 ohm-cm.^{2} However, since no membrane potential was obtained in those experiments, it is unlikely that the electrode had penetrated the egg membrane and this might account for the low estimate. Cole and Curtis (1938) calculated membrane resistances from 0.2 to 10 ohm-cm.^{2} for unfertilized and fertilized *Arbacia* eggs from measurements of single eggs in a small capillary but were forced to discard them because the assumption of no parallel leakage also gave unreasonable values for the membrane capacity. Allen, Lundberg and Runnström (1955) report a resistance of 8.7×10^5 ohms for an egg of *Psammechinus* in a narrow (57 μ) capillary but consider that this may be in error by virtue of a leakage pathway provided between the surface of the egg and the walls of the capillary. They state (p. 178) that in later experiments Lundberg (1955, unpub.) has obtained a value of 1350 ohm-cm.^{2}

Passage of currents of 0.5 to 1.5 μA through the *Asterias* egg, corresponding to current densities of 0.75 to 2.25 mA/cm.^{2}, caused marked decrease of the membrane resistance in 6 eggs. These results need further study, and if the phenomenon is established might yield valuable clues to membrane properties, since the change in resistance was, at least in some eggs, not accompanied by cytolysis. The relatively short pulses used in the experiments did not appear to cause any activation changes such as were reported in the experiments of Allen, Lundberg and Runnström (1955).

It is interesting to note that even with the highest currents employed, the relation between membrane current and voltage was strictly linear both for inward and outward currents. This is not the case in excitable tissues. Outwardly directed currents of relatively low magnitude initiate the changes of membrane potential inherent in the local response and spike and these are associated with a marked drop of membrane resistance, whereas inwardly directed currents tend to increase the resistance (Cole and Curtis, 1941). Thus, eggs of *Asterias*, although they undoubtedly undergo excitation by sperm in the form of the reactions of fertilization, evidently do not respond to electric stimulation in the manner characteristic of electrogenic excitable tissues.

It is of further interest that the maximum quantity of electricity in the square pulses used for the present experiments was about 5×10^{-5} coulomb/cm.^{2} The squid giant axon and other cells, however, are excited by 10^{-8} to 10^{-9} coulomb/cm.^{2} of membrane (Cole, 1949; Grundfest, 1952; Hodgkin, Huxley and Katz, 1952).

Effect of fertilization

If the initial decrease and subsequent increase of the membrane potential in the fertilized egg are consequences of altered ionic flux, the latter change should be reflected as a change of the membrane resistance. The most accurate measurements of this with respect to both magnitude and time course would be provided by study of the high frequency impedance. Such measurements were not done in the present experiments and the values derived from the square pulse technique apply, not to the initial period of fertilization, but to the stage when the fertilization membrane

had been lifted and the membrane potential had reached its steady higher value. At this time the membrane resistance of the two eggs studied before and after fertilization (Fig. 8, Table 4A) was identical with the initial value. This may be taken to indicate that the fertilization membrane is a rather porous structure which offers relatively little impedance to ion movements. Cole (1928), Cole and Spencer (1938) and Cole and Guttman (1942) reported that upon fertilization of sea urchin or frog eggs there was no change in cortical resistance, and our data therefore also support this finding as regards at least a stage a few minutes after fertilization.

However, this constancy poses a dilemma which we have not been able to resolve. It was suggested earlier that the low value of steady membrane potential in the unfertilized egg, and its initial decrease and subsequent increase upon fertilization, might have been caused by alterations in the seal of the egg membrane against the wall of the microelectrode. This assumption would necessitate upward revision of the value of the membrane resistance of the unfertilized egg, perhaps up to double the calculated average of 3100 ohm-cm.^{2}, since the leaks decreasing the membrane potential to about half would be in parallel with the resistance of the membrane. However, the membrane resistance of the eggs seemed to be relatively independent of their steady membrane potentials (Table IVA), and therefore no attempt has been made in the present study to correct the calculated values of R_M. Nevertheless, the possibility remains that the membrane resistance of the unfertilized egg is higher than 3000 ohm-cm.^{2} and that the apparent absence of change after fertilization is fortuitous. The improved sealing of the membrane to the electrode might have increased the leakage resistance greatly and thereby have improved the accuracy of the measurement of R_M. In that case, the actual membrane resistance of the egg may have decreased upon fertilization. More extensive studies will be required to resolve this matter. However, the data, whether the resistance of the membrane is constant or decreases, differ from the finding that the membrane resistance of activated *Fundulus* eggs increases 4- to 7-fold (Kao, 1955). The difference may be explained by the fact that in *Asterias* the egg volume and surface remain nearly constant after fertilization, whereas the activated *Fundulus* egg shrinks markedly. The resultant diminution of surface may therefore lead to closer packing of the ion-permeable units of the plasma membrane (Kao, Chambers and Chambers, 1954), or the effective narrowing of pores in the membrane.

Cole (1938) reported a 100% rise in cortical capacitance of fertilized *Arbacia* and *Hipponöe* eggs and Cole and Spencer (1938) report 300% in *Arbacia*. In the two *Asterias* eggs studied before and after fertilization, the calculated membrane capacity after fertilization remained unaltered in one and decreased in the other. As noted earlier, the accuracy of the measurements of the time constant was not as high as is either desirable or attainable and this matter must be left open subject to future work.

Summary

1. The paper describes electrical characteristics of the egg of the starfish *Asterias forbesii* as measured with a microelectrode penetrating the surface. The study included the effects of fertilizing the egg while the latter was impaled on the electrode.

2. It has been confirmed that penetration of the egg membrane cannot be indicated solely by the seeming visualization of the microelectrode at the center of the egg.

3. A method involving use of two microelectrodes is described for ascertaining penetration of the egg surface. One internal electrode delivers a current pulse and the other records the time course of the resultant membrane IR drop.

4. Contrary to the reports of many earlier investigations on echinoderm and other eggs a potential difference is found upon penetration of the unfertilized egg.

5. The potential difference at the time of penetration amounts to about 60 mv., inside negative, but this soon decreases to lower steady values ranging from − 10 to − 50 mv. in different eggs.

6. Upon insemination of the impaled egg the membrane potential abruptly decreases by 5 to 10 mv. when sperm are seen to have reached the egg, then rises during the ensuing 1 to 2½ minutes, as the fertilization membrane is raised, reaching a new steady value 5 to 20 mv. greater than that of the unfertilized egg.

7. The possible basis of these changes is discussed.

8. The internal K^+ of unfertilized *Asterias* eggs is from 17 to 21 times higher than that of sea water. The sodium determinations, while subject to larger error, indicate a concentration less than 5% that of sea water.

9. The membrane potential of either unfertilized or fertilized eggs decreases when the external K^+ is raised and returns to the original value when the excess K^+ is removed.

10. As in many other kinds of cells the potential is evidently a consequence primarily of the high internal concentration of, and permeability to, K^+, but the magnitude appears less than predicted by the Nernst equation.

11. The membrane resistance of the unfertilized egg averages 3100 ohm-cm.^{2}, but might be higher on the assumption of possible leaks around the microelectrode.

12. The measured resistance is unchanged after fertilization, but, on the assumption of the formation of a tighter electrical seal the actual membrane resistance would be lower.

13. The membrane capacity is of the order of 0.5 μF/cm.^{2}

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